JC10 Rec'd PCT/PTO 07 MAR 2002

FORM PTO-1390 (REV 10-2000) — U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
P/1395-32	10/070597

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CZ00/00064	8 September 2000	8 September 1999

TITLE OF INVENTION
A METHOD OF TRANSGENIC FOWL CONSTRUCTION

APPLICANT(S) FOR DO/EO/US
Pavel TREFIL et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. [X] The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:
    Inventors designation sheet
    12 Drawing Sheets (Figs. 1-12)
    Print EFS Form

EXPRESS MAIL CERTIFICATE

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addresses (mail label EL924372346US in an envelope addressed to: U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202 on March 7, 2002

Dorothy Jenkins
Name of Person Mailing Correspondence

Dorothy Jenkins
Signature

March 7, 2002
Date of Signature

U.S. APPLICATION NO (if known, see 37 CFR 1.5) 10/070597	INTERNATIONAL APPLICATION NO. PCT/CZ00/00064	ATTORNEY'S DOCKET NUMBER P/1395-32

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,040.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO 890.00	
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International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) 710.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00	

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 1,040.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE	
Total claims	3	- 20 =	0	X $18.00	$
Independent claims	1	- 3 =	0	X 84.00	$
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ 280.00	$
TOTAL OF ABOVE CALCULATIONS =				$ 1,040.00	
[ ] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$	
SUBTOTAL =				$ 1,040.00	
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 1,040.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
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b. [ ] Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 15-0700. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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P/1395-32

INVENTORS DESIGNATION SHEET

TITLE: A METHOD OF TRANSGENIC FOWL CONSTRUCTION

PRIORITY CLAIMED UNDER 35 U.S.C. 119:

1. COUNTRY: Czech Republic
APPLICATION NO.: PV 3186-99
DATE OF FILING: September 8, 1999

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APPLICATION INFORMATION

Title Line One:: A METHOD OF TRANSGENIC FOWL CONSTRUCTION
Total Drawing Sheets:: 12
Formal Drawings?:: Yes
Application Type:: Utility
Docket Number:: P/1395-32
Secrecy Order in Parent Appl.?:: No

PRIOR FOREIGN APPLICATIONS

Foreign Application One:: PV 3186-99
Filing Date:: 09-08-1999
Country:: Czech Republic
Priority Claimed:: Yes

Source:: PrintEFS Version 1.0.1

A METHOD OF TRANSGENIC FOWL CONSTRUCTION

Field of the Invention

The present invention relates to a method of transgenic fowl construction using germline spermatogonial cells for transfer of genetic information in fowl strain.

Background of the Invention

Biotechnological research in the field of transgenic fowl is far less successful then the one in the field of the mammals. The main cause of this state is the unique reproductive apparatus of the birds.

In difference to the mammals, the bird´s zygote is not accessible and it is connected with an extraordinarily large yolk sac. The yolk mass prevents identification of pronuclei, in the process of fertilization a polyspermia takes place and it is not possible to determine which protonucleus participated in the embryo formation. Therefore, e.g. the direct microinjection into the protonucleus used in case of mice is nearly impossible in case of fowl. Therefore, in the fowl field, activity was directed to manipulation with the not yet differentiated fowl cells of whatever kind, i.e. blastodermal, primordial gonocytes or germline, spermatogonial cells.

Use of germline, spermatogonial cells in construction of transgenic constructs of mice was disclosed at first by Brinster, L.R. and Avarbock, M.R. (1994) Germline Transmission of Donor Haplotype Following Spermatogonial Transplantation, Proc. Natl. Acad. Sci. USA, 91, 11303-11307.

Virtually, it is a new field of possible germline chimaera production consisting in partial or complete destruction of the reproductive cells in testicles of a creature and in a repeated

re-colonisation of the testicles by germ cells of another creature. Application of this method on fowl was not disclosed.

The first step in mastering the above technique is the testicles sterilization of an acceptor because destruction of such germline spermatogonial cells (sperm progenitors) is not easy. The use e.g. of busulphan is not entirely corresponding, and after its use, only partial sterilization of the donor takes place (see Vick, L., Luke, G., Simkiss, K. (1993) Germline Chimaeras Can Produce Both Strains of Fowl with High Efficiency after Partial Sterilization; J. Reprod. Fertil., 98, 637 - 641. On the other side, use of a higher dose brings about a total retardation of the animal what is unwanted in any case (see Smýkalová, S., Kotrbová, A., Trefil, P. (1998) Effect of Busulphan on Growth and Development of the Chicken Embryos, Vet. Med.-Czech, 43, 105-109).
The other necessary step of a successful application of this method is mastering the transfer of the germline spermatogonial cells into the acceptor, i.e. the transfer of those spermatogonial cells that build in functionally and re-colonize testicles of an acceptor, after their transfer, and subsequently produce fertilizing sperms. At this time this brings many difficulties and ambiguous results.

Summary of the Invention

The above mentioned drawbacks are avoided in case of a method of transgenic fowl construction, preferably by transfer of the germline, spermatogonial cells, according to the present invention which method comprises irradiating the testicles only of an acceptor cock with gamma rays repeatedly, whereafter, foreign germline spermatogonial cells of a donor cock are implanted into the undamaged sterile testicles of said acceptor

cock within an interval of 60 days to 2 years after the last irradiation dose of said testicles, whereby, said testicles continue in producing fertilizing sperms that store the whole genetic information of the donor cock. After insemination of hens by the so produced sperms, transgenic fowl chickens are hatched and provided ½ genetic information by said donor cock.

The method according to the present invention is preferably carried out so that the testicles of the acceptor cock are irradiated with gamma rays applying absorbed dose of at least 8 Gy 3-times to 9-times, always within an interval of 3 to 9 days.

Preferably, the method according to the present invention is carried out so that the testicles of the acceptor cock are irradiated with gamma rays using the absorbed dose 8 Gy 5-times in a week interval.

In contradiction to the other disclosed techniques, in the case of this irradiation, the original germline, spermatogonial cells are completely destroyed in the testicles of the acceptor cock, whereafter, the acceptor cook is unable to produce sperms, whereby the testicle structure including the Sertoli's cells remains preserved for the subsequent implantation of foreign germline spermatogonial cells provided by a donor cock. The so implanted germline spermatogonial cells continue in producing sperms that are able to fertilize and that store the genetic information of the germline spermatogonial cells of the donor cock (see the Diagram in Fig. 2).

Production of transgenic fowl by the method according to this invention is easy and provides results to 100 %, because the spermatogonial cells of the donor cock can be transfected by the

required genetic information and so, after a successful implantation of said cells into the testicles of the acceptor cock a transgenic cock is produced, which transgenic cock will transfer to ½ the genetic information to his progeny by insemination or by natural breeding.

The fact itself that this method of transferring germline spermatogonial cells from one creature to another one is functional is extremely valuable and apart from this, it is extremely interesting especially in relation with the creation of transgenic fowl. The germline spermatogonial cells can be easily transfected in vitro by foreign genetic information before the implantation proper, which information will then be transferred to ½ to the offspring in the F1 generation. This will probably open a real way how to use fowl (fowl oviduct) as a bioreactor for production of specific proteins valuable e.g. for the pharmaceutical industry.

Therefore, a great number of tests were carried out to verify the method according to this invention, whereby, some of them are described below as examples with reference to the attached graphs and drawings.

Brief Description of the Drawings

Figure 1 shows a photograph of seminiferous tubulus (Sem.t.) of repeatedly (5-times 8 Gy dose) irradiated of testes of an acceptor cock. Seminiferous epithelium are lined only by Sertoli cells(S.C.) and interstitial tissue consisting of Leydig cells(L.C.). Germline, spermatogonial cells are not visible (PAS stain, enlarged 400-times).

Figure 2 shows a diagram of the method, whereby, a Minorca black cock (ii,EE,b/b) is the donor cock 1, a Leghorn white (II) cock is the acceptor cock 2 which acceptor cock 2 is irradiated by a 8 Gy absorbed dose 5-times to stop production of own sperms. The acceptor cock 2 is then colonized by spermatogonial cells in suspension 4. The acceptor cock then inseminates a Leghorn barred hen 3 (ii,ee,B/-) to produce crossbreeds, i.e. a (ii,Ee,B/b) barred male chicken 5 and a (ii,Ee,b/-) black female chicken 6.

Figure 3 shows a graph of relation between sperms concentration (x1000/ml)(y-axis) and days elapsed after an irradiation (x-axis) 5-times by a 8 Gy absorbed dose.

Figure 4 shows a graph of relation between motility (y-axis) and days (x-axis) elapsed after irradiation 5-times by a 8 Gy absorbed dose.

Figure 5 shows a graph of relation between sperms concentration (x1000/ml)(y-axis) and days elapsed after irradiation (x-axis) by a 18 Gy absorbed dose.

Figure 6 shows a graph of relation between motility (y-axis) and days (x-axis) elapsed after irradiation by a 18 Gy absorbed dose.

Figure 7 shows a graph of relation between sperms concentration (x1000/ml)(y-axis) and days elapsed after irradiation (x-axis) by a 22 Gy absorbed dose.

Figure 8 shows a graph of relation between motility (y-axis) and days elapsed after irradiation (x-axis) by a 22 Gy absorbed dose.

Figure 9 shows a graph of relation between sperms concentration (x1000/ml) (y-axis) and days elapsed after irradiation (x-axis) by a 26 Gy absorbed dose.

Figure 10 shows a graph of relation between motility (y-axis) and days elapsed after irradiation (x-axis) by 26 Gy absorbed dose.

Figure 11 shows a graph of relation between sperms concentration (x1000/ml) (y-axis) and days elapsed after irradiation (x-axis).

Figure 12 shows a graph of relation between motility (y-axis) and days elapsed after irradiation (x-axis).

For the comparison of spermatozoa motility and spermatozoa concentration (from Fig.3 to Fig.12) between treated groups, the linear regression model was used-Baloui(1966), Likeš and Machek(1983). From the slope of on the gradient line it is possible to characterize the changes of the values in the parameters.

Spermatozoa motility of was subjectively estimated according to Trefil, 1995.Selection of chicken sires for semen production in cages. Book of abstracts of the 46th Annual Meeting of the European Association for Animal Production, Prague, September,165. Very good motility has a no. 5 rating and very poor motility no.1.

Statistically, there was no difference between the control group (Fig.12) and the groups of cocks irradiated with 18 Gy (Fig.6) and 22 Gy (Fig.8). Groups treated with a single dose of 26 Gy (Fig.10) and 5 x 8 Gy (Fig.4) doses differed significantly in their linear gradients compared with the control group and those administered with 18 Gy and 22 Gy.

From a statistical point of view, the linear gradients of the spermatozoa concentration did not differ between the control group (Fig.11) and groups irradiated with single 18 (Fig.5) and 22Gy (Fig.7) doses. However, a significant difference was shown with the single 26Gy group (Fig.9) and the group repeatedly irradiated by 5 doses of 8Gy (Fig.3) compared to 18Gy, 22Gy and control groups.

The group treated with one dose of 26Gy never attained zero spermatozoa concentrations.

Detailed Description of the Invention

Example 1

Complete sterilization of an acceptor cock:

A mature cock of the Leghorn white inbred strain (white colour of feathers is caused by the dominant allele II in locus I) producing ejaculate was fixed in a box so that it was possible to carry out an irradiation with gamma rays directed on the side of his body exactly to an area of the testicle´s size. The

instrument, model Theratron T1000,with isotope ^{60}Co was suitable for this purpose. Using this instrument testicles of this cock were irradiated 5-times in a week minimally by the absorbed dose 8 Gy. Without producing any adverse effects on the cock health and breeding condition, about 90 days after the last irradiation the cock lost the ability to produce sperms permanently and after this period his testicles were ready to be colonized by the germline spermatogonial cells produced by the donor cock, see the graphs in Fig. 3 and Fig. 4 and the photograph in Fig. 2.

Preparation and application of germline spermatogonial cells of the donor cock:

A mature cock of the Minorca black inbred strain (black color of feathers is caused by the recessive allele ii) producing ejaculate was subjected to a tissue sampling by biopsy from his testicles which sample contained his germline spermatogonial cells. The cells were immersed into the M 199 Sigma medium (containing expressed by weight: 10 % of fetal bovine serum, 2 % of chicken serum, 1 % of pyruvate sodium, 1 % of gentamycin), whereby, the dilution ratio with this medium was 1:1. Cell incubation was carried out in standard conditions, i.e. carbon dioxide content 5 % by weight, temperature 40 °C. Spermatogonial cells could be transfected with any foreign DNA during this incubation. Thereafter, the total volume 200 microlitres of the germline spermatogonial cells suspension was applied by means of a syringe into each irradiated testicle of the acceptor cock at the time when the irradiated cock ceased to produce any ejaculate, i.e. 85 to 100 days after the last irradiation dose.

Functional production of ejaculate after a transfer of the germline spermatogonial cells of the donor cock:

The irradiated acceptor cock of the Leghorn inbred strain with non-functional testicles started to produce ejaculate after application of foreign germline spermatogonial cells of the donor cock of the Minorca black inbred strain.

Biological testing of the transferred germline spermatogonial cells of the donor cock:
Having carried out insemination of a hen of the Leghorn barred inbred strain (the barred color is caused by recessive allele ii by piebald gene bound to sex B/-) by ejaculate taken from the irradiated cook of the Leghorn white inbred strain, which cock is acceptor of foreign germline spermatogonial cells of the donor cock of the Minorca black inbred strain, the inseminated eggs were put into an incubator and after 21 days of incubating only black chicken hatched.
The black chicken are a sound proof that the implantation and colonization of the germline spermatogonial cells of the donor cock was successful because if only black or barred chicken hatch, in fact it indicates that the dominant allele II is not present and that only the recessive allele ii is present, i.e. that only functional sperms of the cock with recessive feather color ii are present, i.e. in this case of those with the black feather color, see Fig. 2.

Example 2
Incomplete sterilization of the acceptor cock:
A mature cock of the Leghorn white inbred strain (white feather colour caused by dominant allele II in locus I) producing ejaculate was fixed for irradiation in the same way as in Example 1. Testicles of this cock were irradiated by means of the irradiation instrument by a one-time absorbed dose of 18 Gy. Without deteriorating the health state and the breeding condition of the cock, the cock did not loose the ability to

produce sperms permanently (in comparison to check, see the graphs in Figures 11 and 12), on the contrary, after 150 days the concentration (see the graph in Fig. 5) and the motility (see the graph in Fig. 6) returned to normal values before the irradiation and the cock testicles were not prepared to be colonized by germline spermatogonial cells of the donor cock.

Example 3

Incomplete sterilization of an acceptor cock:

A mature cock of the Leghorn white inbred strain (white colour of feathers is caused by the dominant allele II in locus I) producing ejaculate was fixed in a box so as in Example 1 during irradiation. Testicles of this cock were irradiated with a one-time absorbed dose 22 Gy provided by the same irradiation instrument.

Without producing any adverse effects on the cock's health and breed condition, during 200 days of monitoring, the cock did not lose the ability to produce sperms permanently. Conversely, after 160 days, the concentration (see graph in Fig. 7) and motility (graph in Fig. 8) were a little bit lower, but they returned to the original level existing before the irradiation and the cock´s testicles were not prepared to be colonized by germline spermatogonial cells of the donor cock.

Example 4

Incomplete sterilization of the acceptor cock:

A mature cock of the Leghorn white inbred strain (white color of feathers is caused by the dominant allele II in locus I) producing ejaculate was fixed in a box so as in Example 1 during irradiation. Testicles of this cock were irradiated with a one-time absorbed dose 26 Gy provided by the same irradiation instrument. This absorbed dose influenced health state of this cock negatively. The cock´s skin was slightly swollen and

reddish in the irradiation place, the cock was tired for several days after the irradiation. The cock lost ability to produce sperms not sooner then nearly after 160 days, (see graph in Fig. 9), but production of ejaculate continued at a very low level and the sperms have shown certain motility (see graph in Fig. 10). It was obvious that this absorbed dose was rather too high, it deteriorated health state of the cock, but considering that the ejaculate production continued, this absorbed dose was still insufficient and the cock testicles were not yet prepared for colonization by the germline spermatogonial cells of the donor cock.

Industrial Use

By repeated irradiation of the acceptor cock´s testicles with gamma rays and by implanting foreign germline spermatogonial cells of the donor cock into the so treated undamaged but sterile testicles of the acceptor cock, sperms able to fertilize that store all genetic information of the donor cock are repeatedly produced by this acceptor cock. After insemination of hens by the sperms so generated, transgenic fowl is produced which fowl is provided to ½ with the genetic information of the donor cock.

Method according to the present invention will find industrial use in fowl breeding and biotechnological industries.

Production of transgenic fowl by this method is easy because the spermatogonial cells of the donor cock can be transfected by the necessary genetic information and so after a successful implantation of said cells into testicles of a acceptor cock a transgenic cock is so produced, which cock after insemination of

hens by his ejaculate or by natural breeding will pass to ½ the genetic information of the donor to his offspring.

Claims

1. A method of transgenic fowl construction using germline spermatogonial cells for transfer of genetic information in fowl strain, characterized in that the testicles only of an acceptor cock are irradiated with gamma rays repeatedly and externally, whereafter, the so treated preserved but now sterile testicles of this acceptor cock are colonized by foreign germline spermatogonial cells of a donor cock, which cells then produce sperms that store all genetic information of the donor cock, whereby, the sperms are able to fertilize hens after their insemination, whereby, transgenic fowl offsprings provided to ½ with the donor cock genetic information are breed.

2. A method according to Claim 1 characterized in that said testicles of said acceptor cock are irradiated with gamma rays up to the absorbed dose of at least 8 Gy, what is carried out 3-times to 9-times, always in 3 to 9 day intervals.

3. A method according to Claim 1 characterized in that said testicles of said acceptor cook are irradiated with gamma rays up to the absorbed dose 8 Gy always in week intervals.

(12) INTERNATIONAL [redacted]CATION PUBLISHED UNDER THE PATE[redacted]OPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/17344 A3

(51) International Patent Classification[7]: A01K 67/027

(21) International Application Number: PCT/CZ00/00064

(22) International Filing Date:
8 September 2000 (08.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PV 3186-99 8 September 1999 (08.09.1999) CZ

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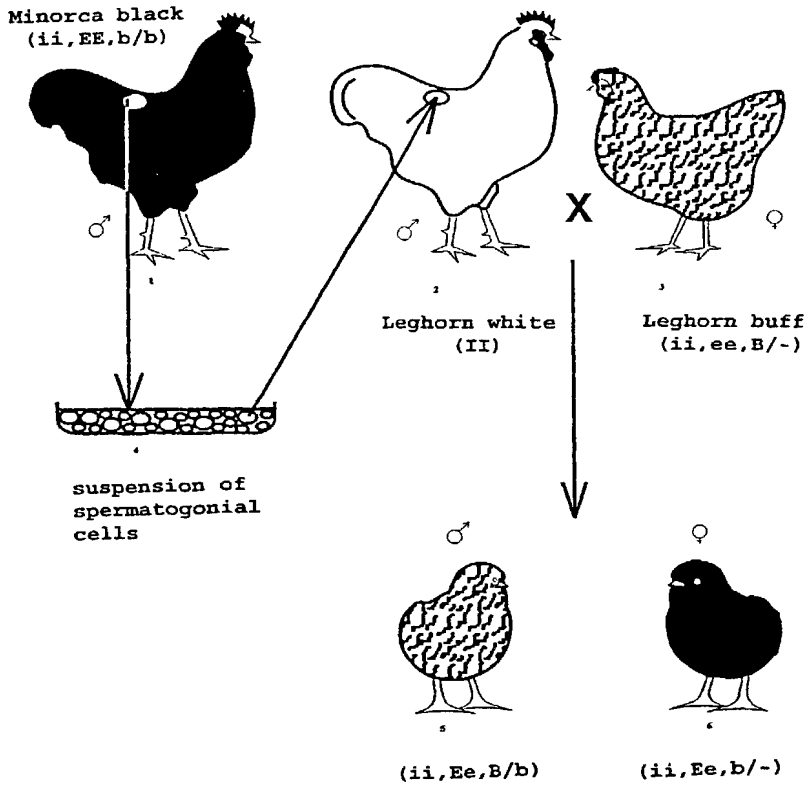
(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

[Continued on next page]

(54) Title: A METHOD OF TRANSGENIC FOWL CONSTRUCTION



(57) Abstract: A method of transgenic fowl construction using germline spermatogonial cells for transfer of genetic information in fowl strain, which method is carried out so that the testicles only of an acceptor cock are irradiated with gamma rays up to the absorbed dose 8 Gy in one irradiation repeatedly and externally. Thereby, the original germline spermatogonial cells in the testicles of the acceptor cock are destroyed, which acceptor cock is then not able to produce sperms, whereby, the testicles structure, including the Sertoli's and Leydig's cells remains preserved for implantation of foreign germline spermatogonial cells of a donor cock. The new implanted germline spermatogonial cells then continue in production of sperms. The sperms are able to fertilize and that store all genetic information of the germline spermatogonial cells of the donor cock. The implantation is carried out after 50 days and more after the last irradiation.

WO 01/17344 A3

A photography of the seed producing ducts after repeated (5-times 8 Gy dose) irradiation of testicles of an acceptor rock. In the sperm producing ducts only the Sertoli's cells are visible, the germline spermatogonial cells are not visible (dyed by Pas, enlarged 400-times).

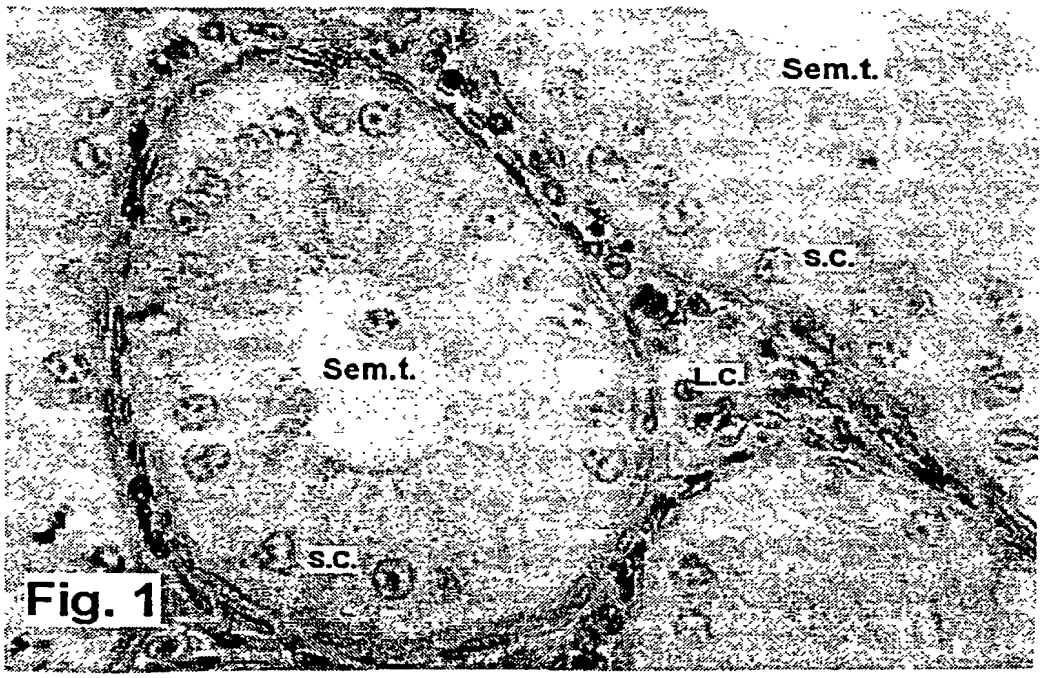


Fig. 1

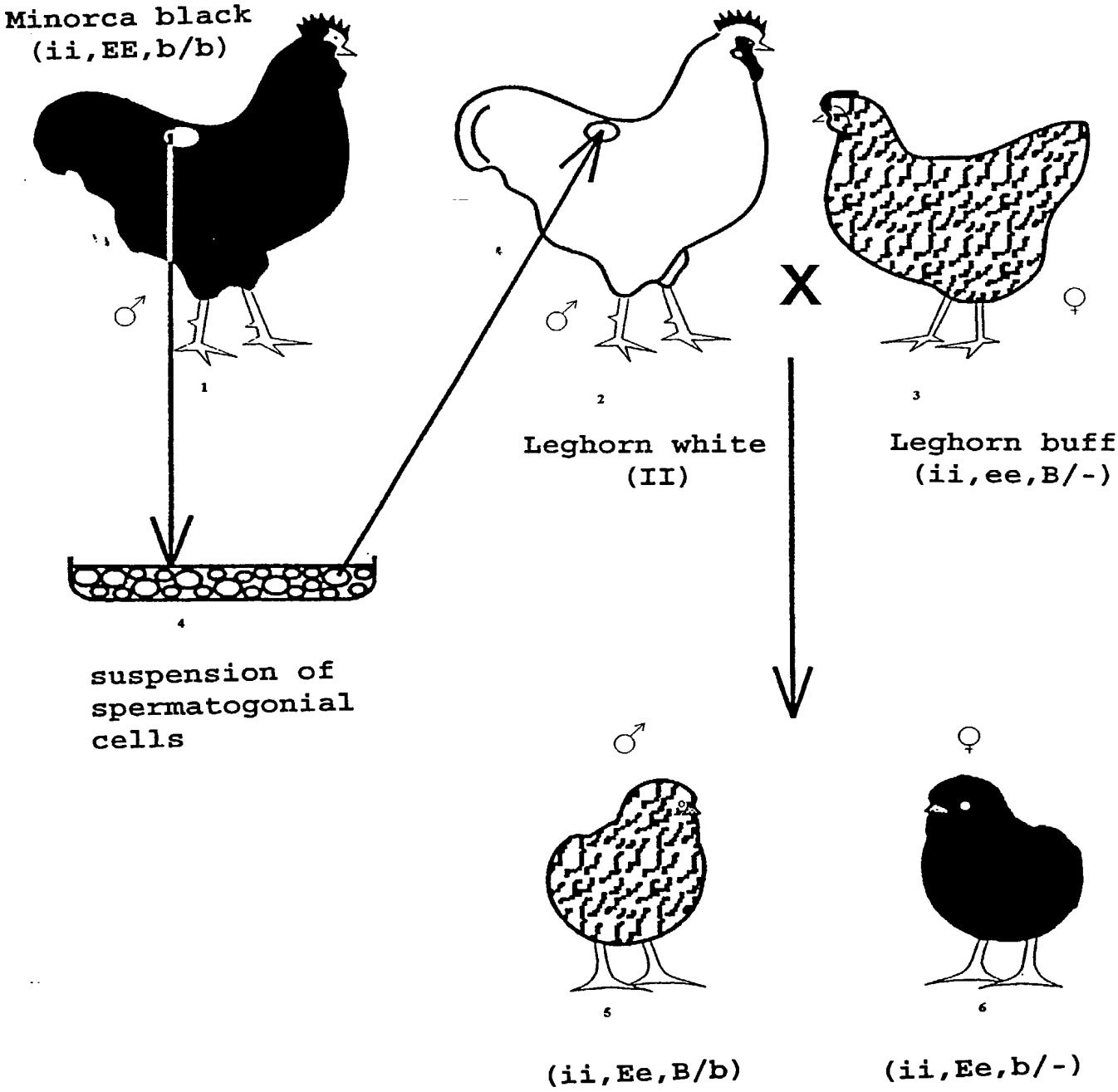

Minorca black
(ii,EE,b/b)
♂
1
♂
2
X
♀
3
Leghorn white
(II)
Leghorn buff
(ii,ee,B/-)
4
suspension of
spermatogonial
cells
♂
♀
5
6
(ii,Ee,B/b)
(ii,Ee,b/-)

Fig. 2

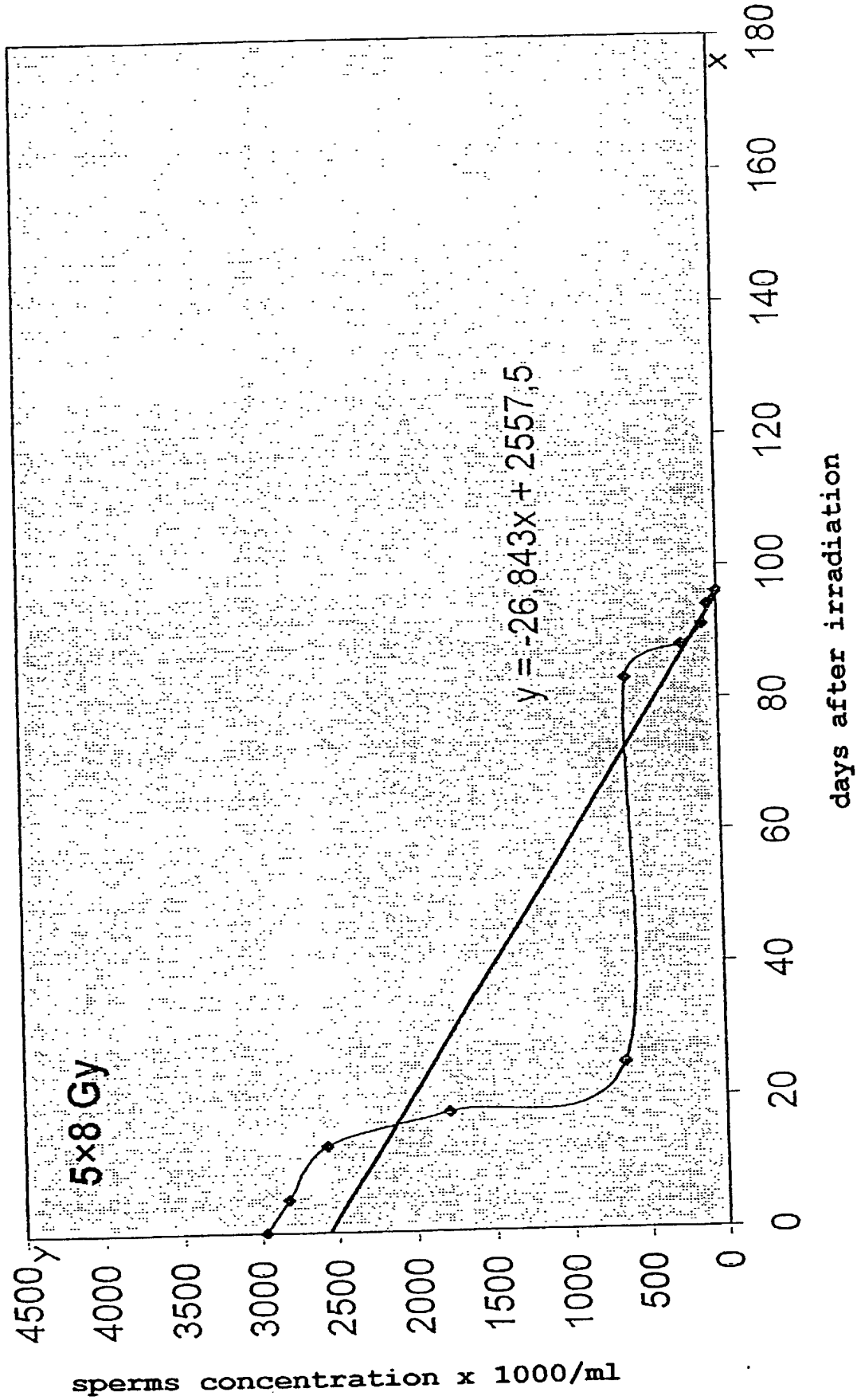

5x8 Gy
y = -26,843x + 2557,5
sperms concentration x 1000/ml
days after irradiation
y
x
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
160
180

Fig. 3

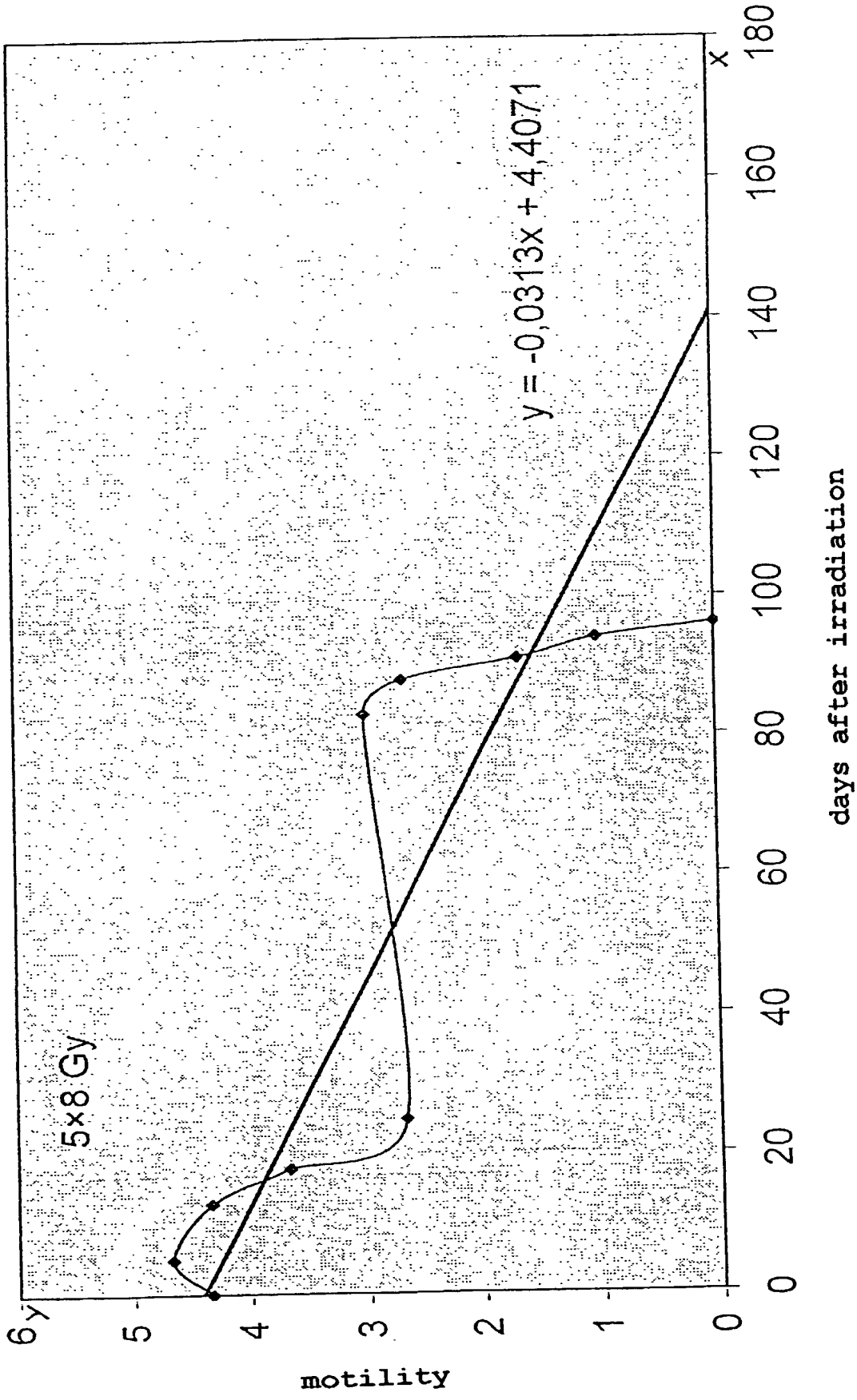

y = -0,0313x + 4,4071
5×8 Gy
motility
days after irradiation
0
1
2
3
4
5
6
y
0
20
40
60
80
100
120
140
160
180
x

Fig. 4

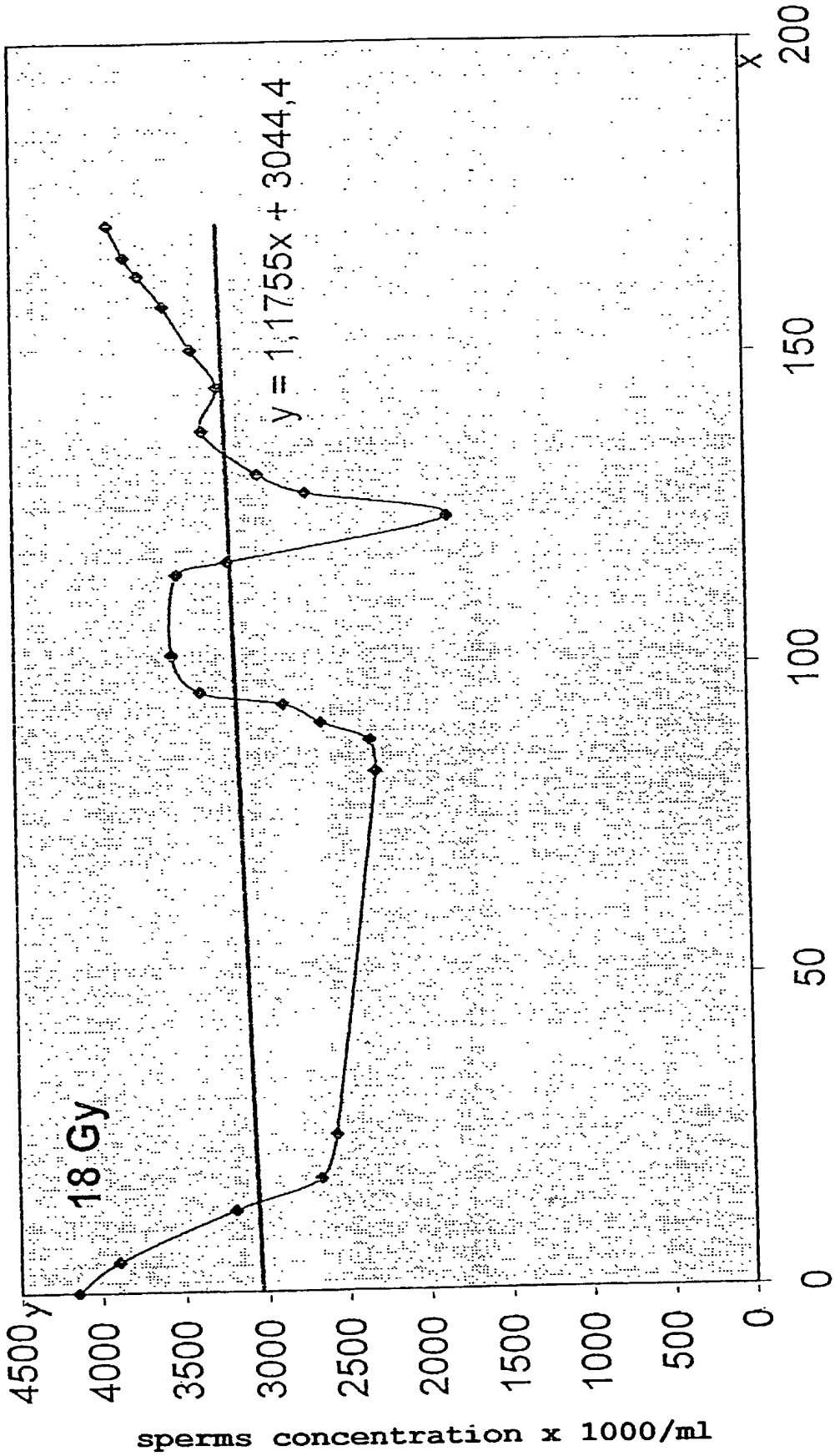

y = 1,1755x + 3044,4
18 Gy
y
x
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
50
100
150
200
sperms concentration x 1000/ml
days after irradiation

Fig. 5

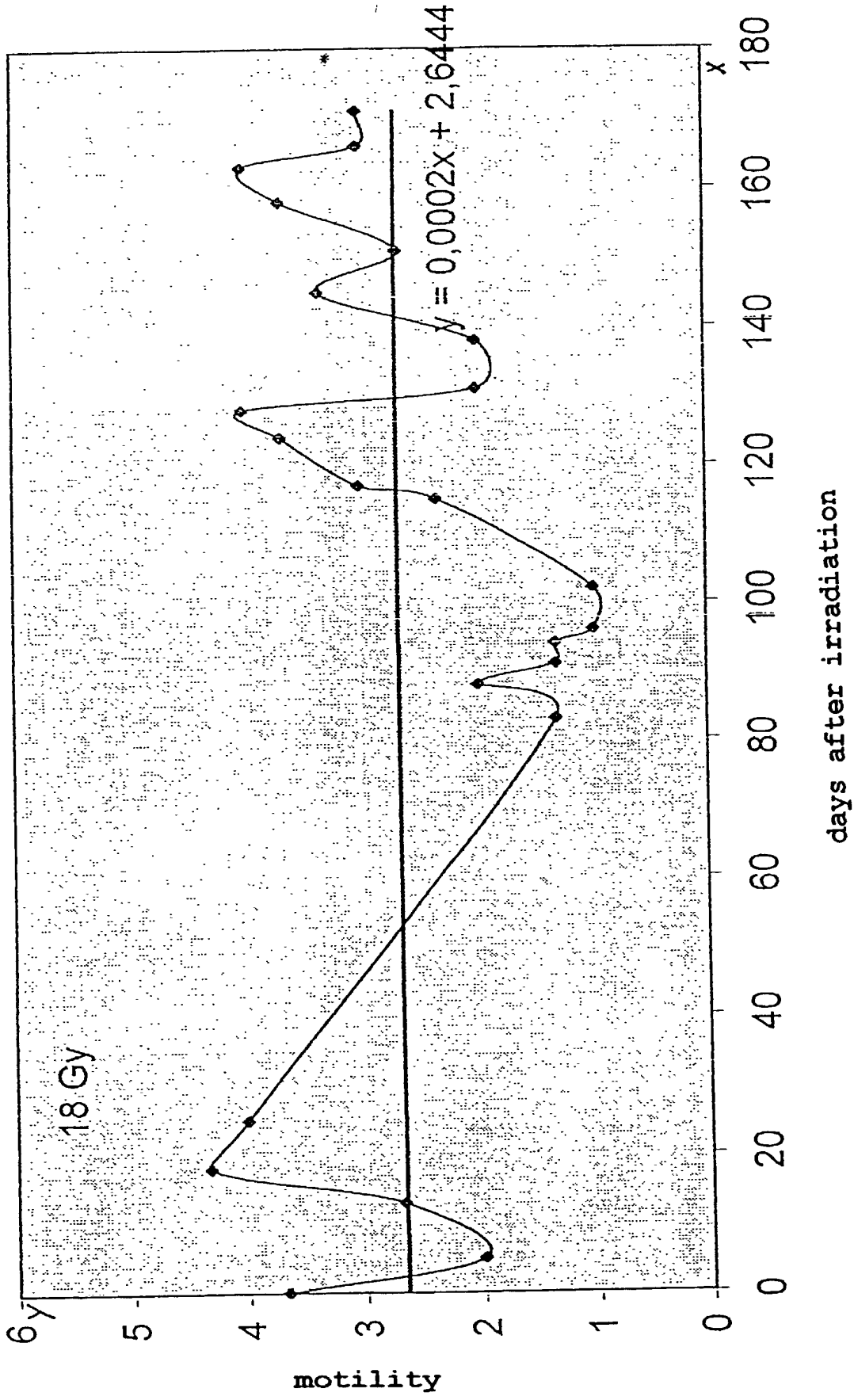

y = 0,0002x + 2,6444
18 Gy
y
x
motility
days after irradiation
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180

Fig. 6

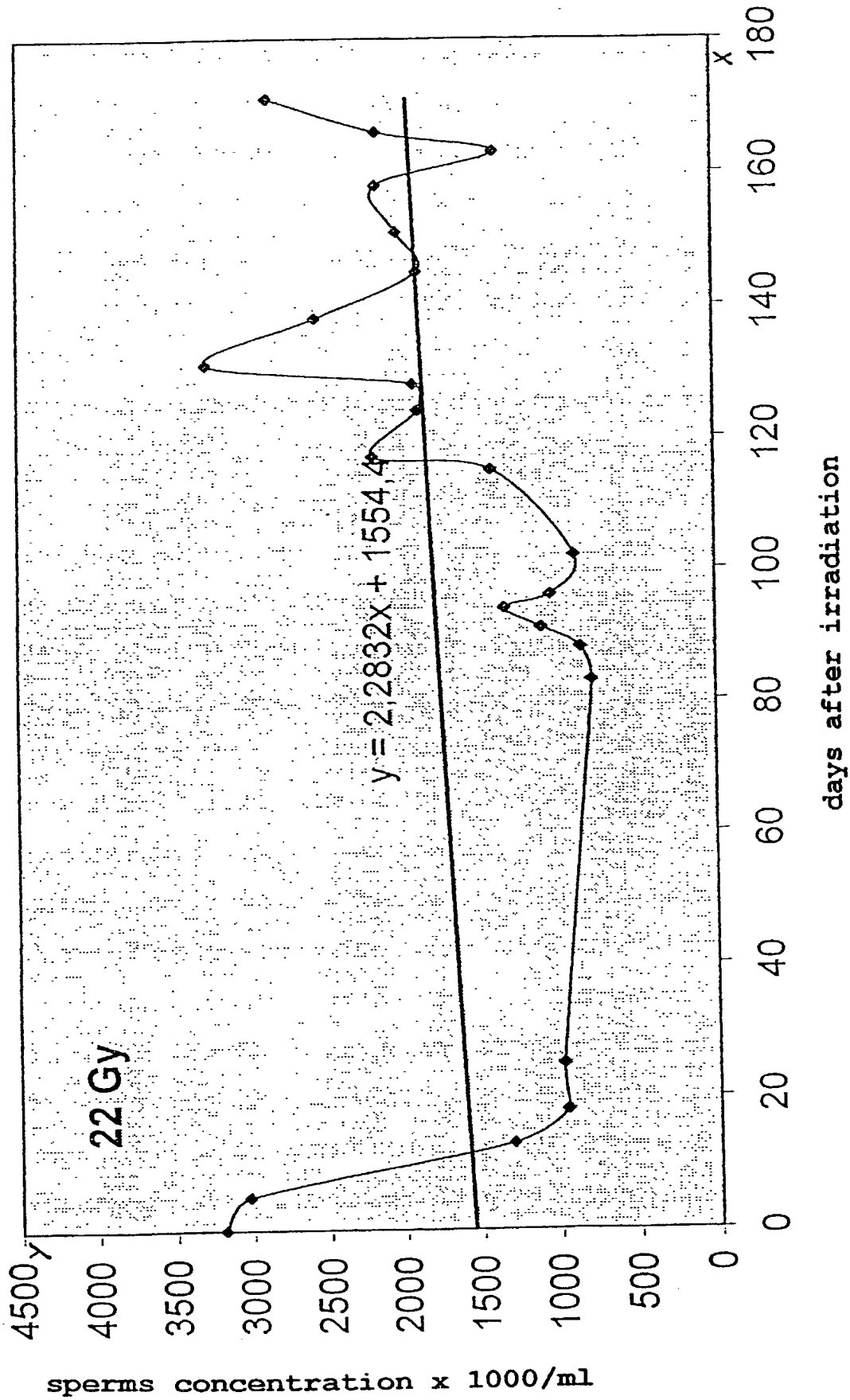

22 Gy
y = 2,2832x + 1554,4
sperms concentration x 1000/ml
days after irradiation
y
x
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
160
180

Fig. 7

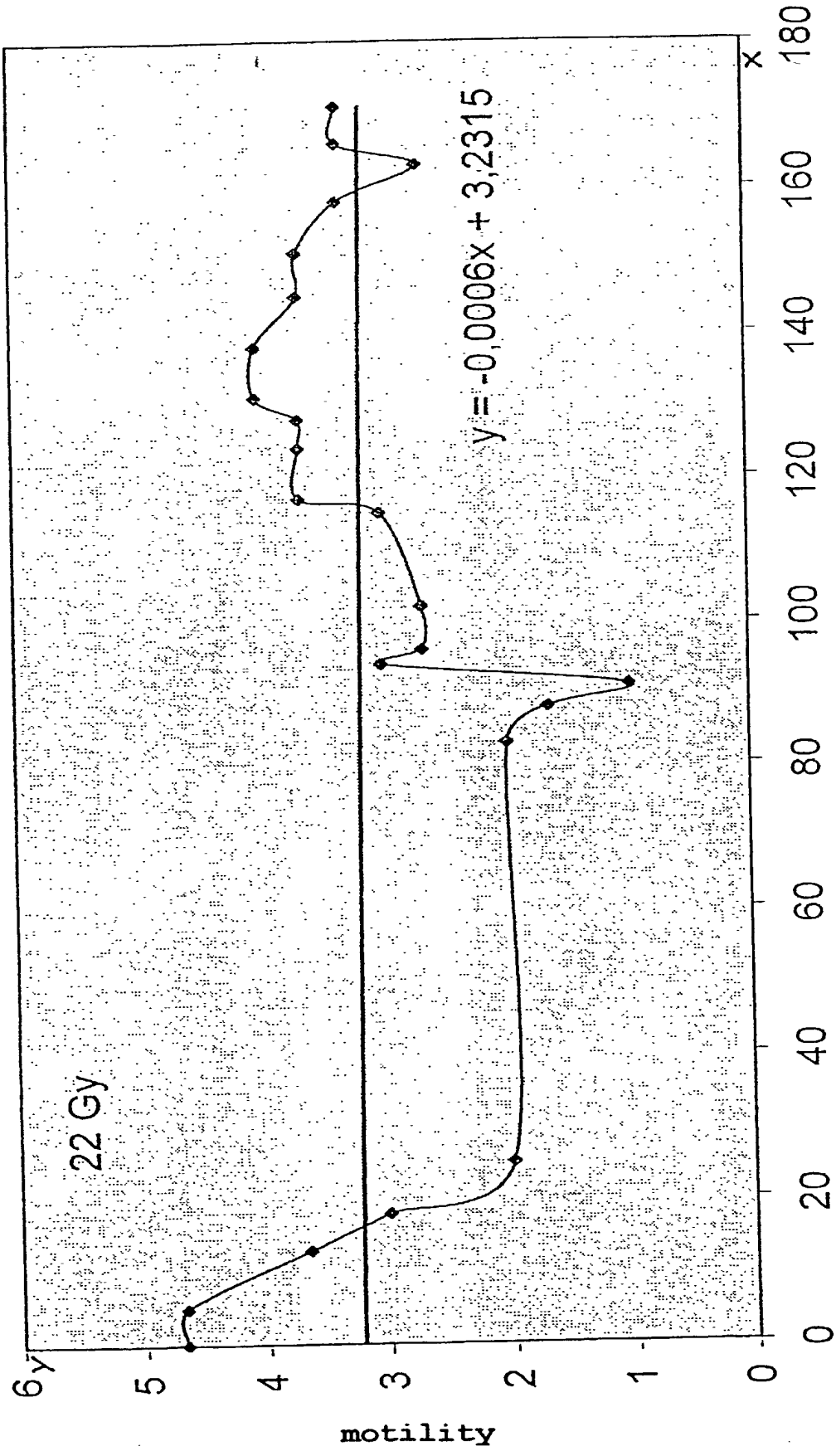

y = -0,0006x + 3,2315
22 Gy
6y
motility
days after irradiation
0
1
2
3
4
5
0
20
40
60
80
100
120
140
160
180
x

Fig. 8

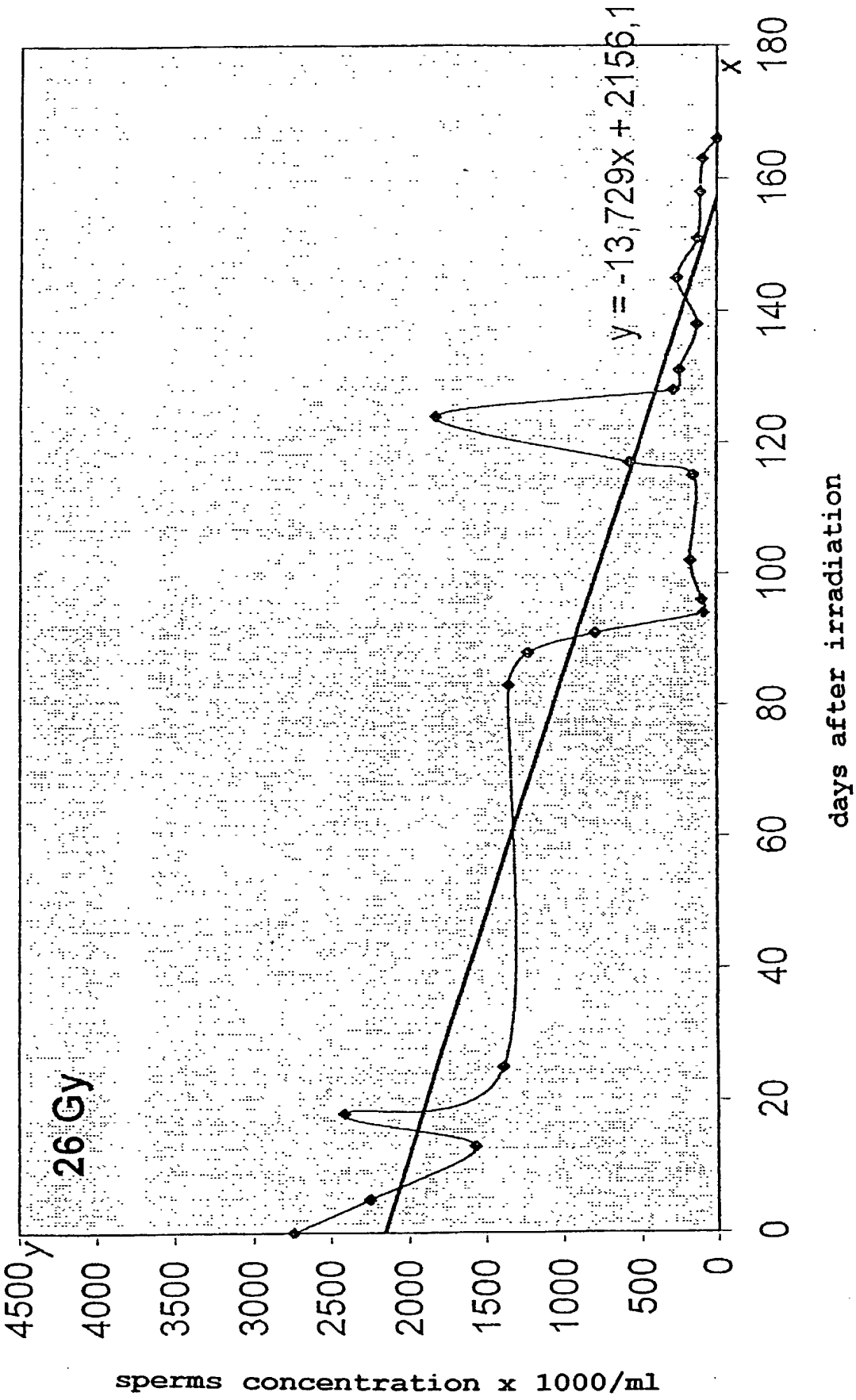

y = -13,729x + 2156,1
26 Gy
y
x
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
160
180
sperms concentration x 1000/ml
days after irradiation

Fig. 9

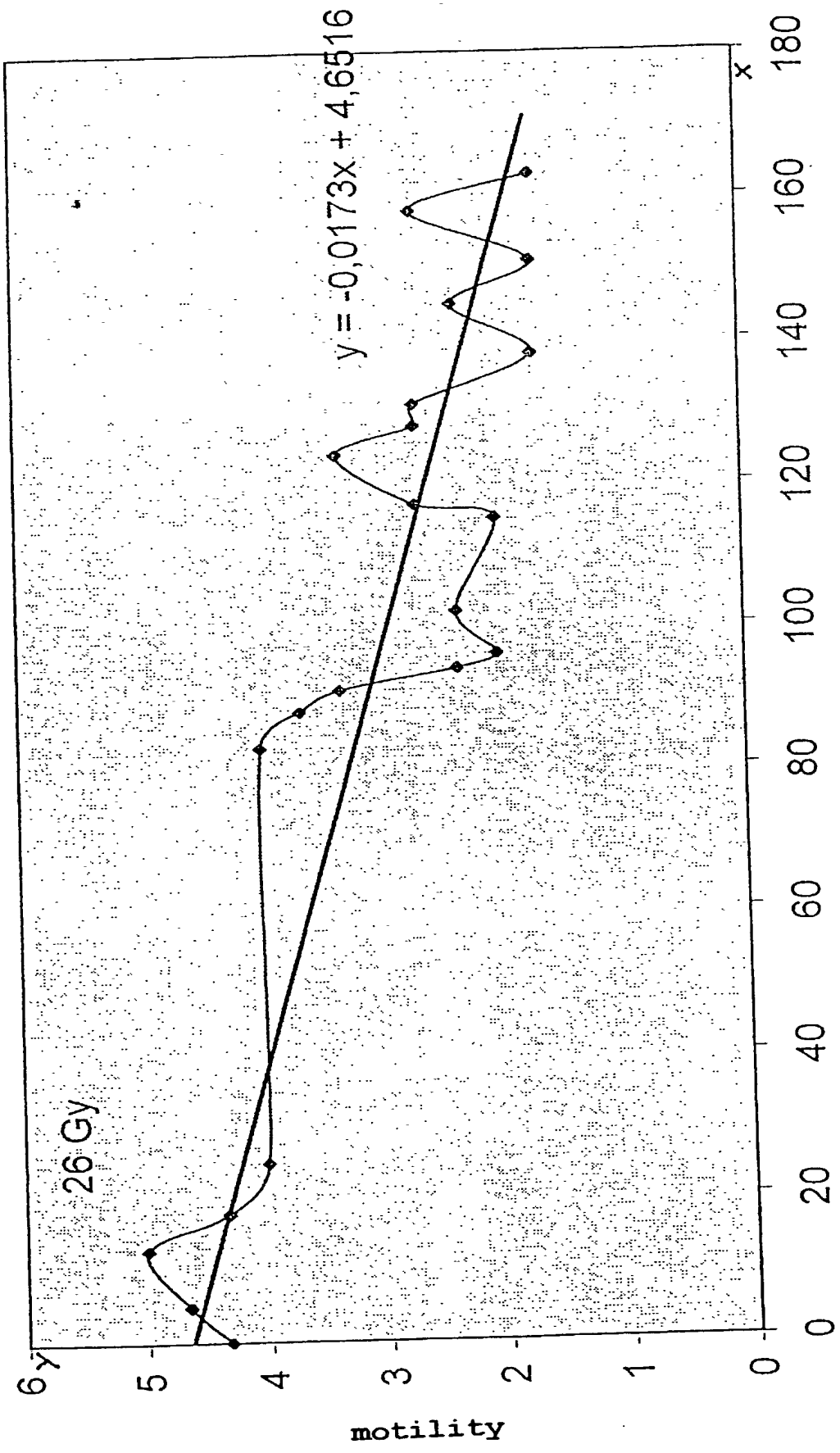

y = -0,0173x + 4,6516
26 Gy
motility
days after irradiation
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180
x
y

Fig. 10

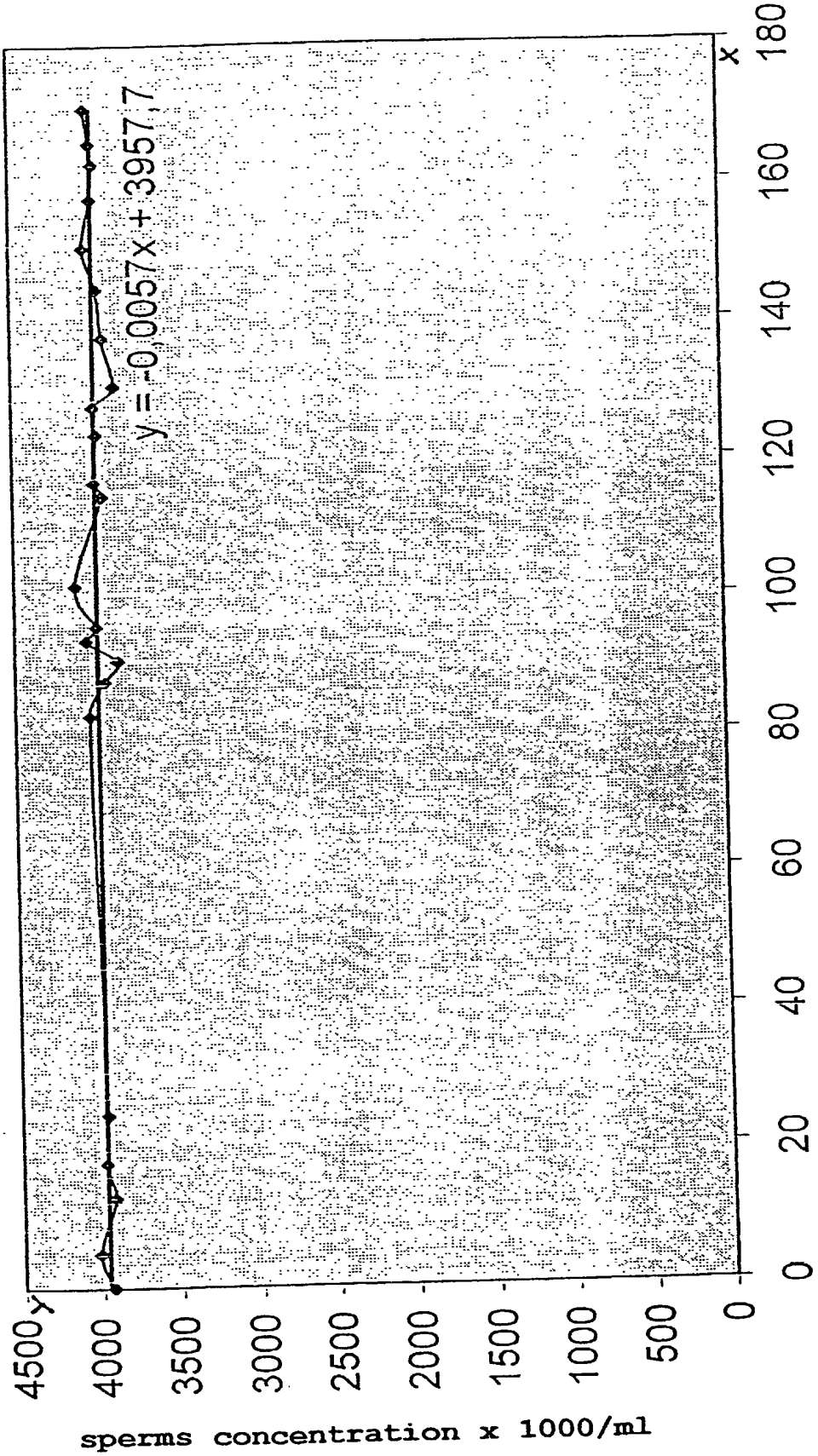

y = -0,0057x + 3957,7
sperms concentration x 1000/ml
days after irradiation
4500
4000
3500
3000
2500
2000
1500
1000
500
0
y
x
0
20
40
60
80
100
120
140
160
180

Fig. 11

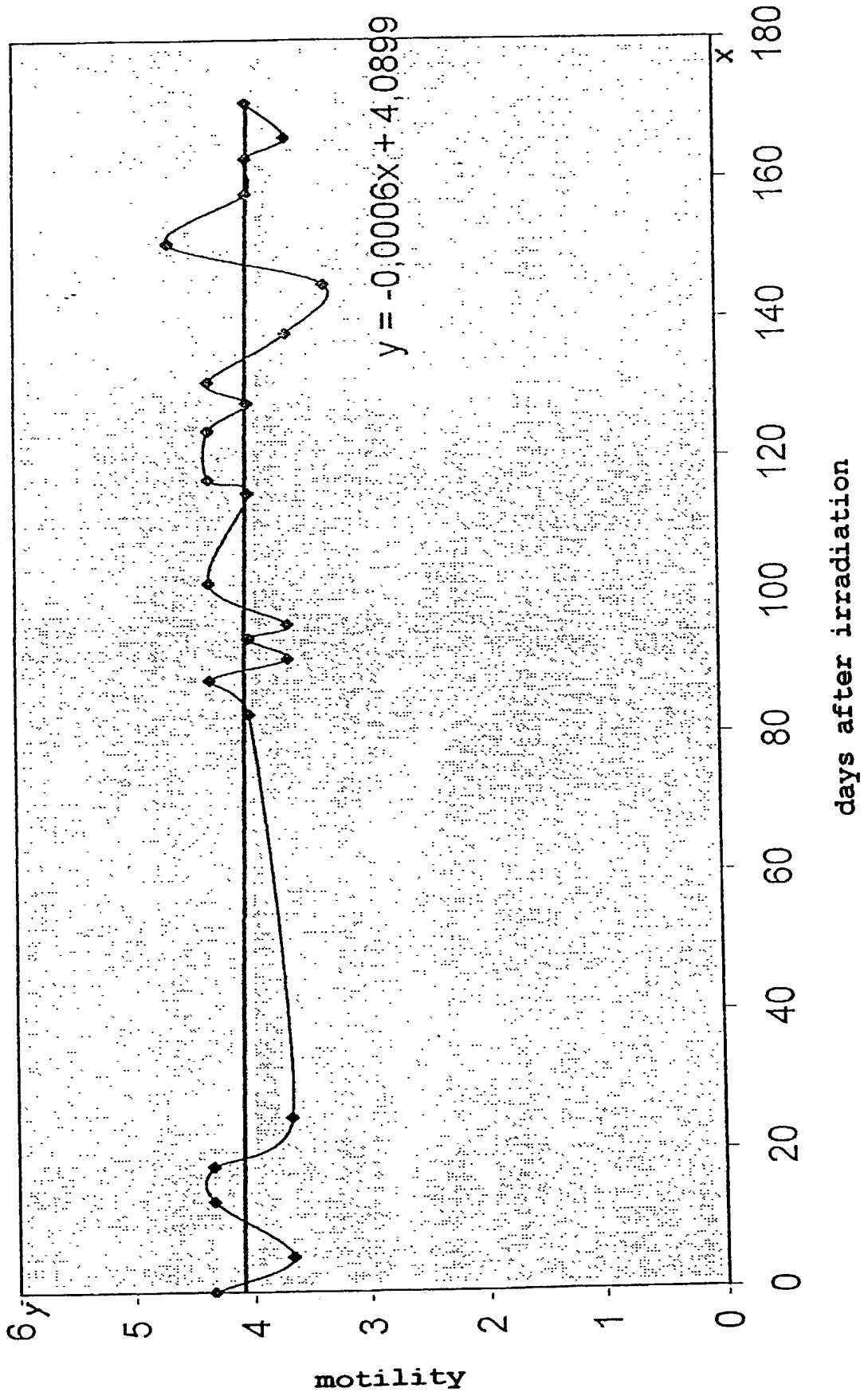

y = -0,0006x + 4,0899
y
x
motility
days after irradiation
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180

Fig. 12

UNITED STATES OF AMERICA
COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

OFGS FILE NO.
P/1395-32

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; that I verily believe that I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named) of the subject matter which is claimed and for which a patent is sought on the invention entitled

A METHOD OF TRANSGENIC FOWL CONSTRUCTION

the specification of which is attached hereto, unless the following box is checked
☒ was filed on March 7, 2002 as United States patent Application Number or PCT International patent application number _____ and was amended on _____ (if any)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above

I acknowledge the duty to disclose all information known to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim priority benefits under Title 35, United States Code §119 of any foreign application(s) for patent or inventor's certificate or United States provisional application(s) listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed

Prior Foreign or Provisional Application(s)

COUNTRY	APPLICATION NUMBER	DATE OF FILING *(day, month, year)*	PRIORITY CLAIMED UNDER 35 U S C 119
Czech Republic	PV 3186-99	8 September 1999	YES X NO ___
			YES ___ NO ___
			YES ___ NO ___

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application

UNITED STATES APPLICATION NUMBER	DATE OF FILING *(day, month, year)*	STATUS *(patented, pending, abandoned)*

I hereby appoint customer no. 2352 OSTROLENK, FABER, GERB & SOFFEN, LLP, and the members of the firm, Samuel H. Weiner - Reg. No. 18,510; Jerome M Berliner - Reg No 18,653; Robert C. Faber - Reg No 24,322, Max Moskowitz - Reg No 30,576, James A Finder - Reg No. 30,173; William O Gray, III - Reg No 30,944, Louis C. Dujmich - Reg No. 30,625, and Douglas A. Miro - Reg No 31,643, as attorneys with full power of substitution and revocation to prosecute this application, to transact all business in the Patent & Trademark Office connected therewith and to receive all correspondence.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon

1-00

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INVENTOR'S SIGNATURE: Alena Kotrbová
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